IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicants:	**Baker, B.F., *et al.***	Confirmation No.:	**7033**
Serial No.:	**10/701,265**	Group Art Unit:	**1635**
Filed:	**November 4, 2003**	Examiner:	**Jennifer S. Pitrak**
Title:	**Chimeric Oligomeric Compounds And Their Use In Gene Modulation**		

Commissioner for Patents
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DECLARATION OF DAVID COREY

I, David Corey, Ph.D., residing at 4516 South Versailles Ave, Highland Park, TX 75205, hereby declare that:

1. I currently hold the position of Professor in the Departments of Pharmacology and Biochemistry at the University of Texas Southwestern Medical Center in Dallas Texas.

2. I earned a B.A. in Chemistry from Harvard University in 1985 and a Ph.D. in Chemistry from the University of California at Berkeley in 1990. I have been on the faculty of UT Southwestern since 1992. The National Institutes of Health, The Howard Hughes Medical Institute, The Robert A. Welch Foundation, and numerous other public and private funding organizations have provided financial support for my research. I have published over 86 peer-reviewed research articles and 38 book chapters or journal review articles. I currently serve on editorial boards of several peer-reviewed journals, including Nucleic Acids Research, Oligonucleotides, Cancer Research, RNA and Gene Silencing, Chemical Biology of Drug Design, Tetrahedron, and Tetrahedron Letters. I am an inventor on one issued U.S. Patent and seven pending patent applications. My curriculum vitae is attached hereto as Appendix A.

3. I understand that the present application is assigned to Isis Pharmaceuticals, Inc. I served as a consultant for Isis Pharmaceuticals from 2002-2005. I have also been an expert witness for Isis Pharmaceuticals on two previous occasions and have collaborated with scientists from Isis Pharmaceuticals on several different scientific investigations. I understand that Alnylam Pharmaceuticals also has an interest in the pending application. Alnylam has provided research support to my laboratory and we are currently collaborating on three different projects. Alnylam has also licensed unrelated intellectual property generated in my laboratory at UT Southwestern.

4. I have read and understand pending United States patent application No. 10/701,265, including the office actions dated October 18, 2006; January 9, 2008; and October 31, 2008, together with the replies and amendments filed in response thereto. I have also read the office action dated February 19, 2009, and the response to that office action, including claim amendments.

5. The claims now pending in this patent application are directed to duplexes comprising two separate chemically synthesized oligonucleotides hybridized to one another. In the broadest claim, claim 120, each oligonucleotide of the duplex is from 17-25 nucleosides in length; the two oligonucleotides are fully complementary to one another, and one of them is also fully complementary to a messenger RNA and has a gapmer motif, wherein the gap region comprises RNA nucleosides and each wing comprises modified nucleosides having a modification at the 2'-position of the sugar ring.

6. I have reviewed and understand the cited references: Lee et al., Cell, 1993, vol. 75, p 843-854 (Lee); Manche et al., Mol and Cell Bio, 1992, vol. 12, p 5238-5248 (Manche); Baracchini et al., US 5,801,154 (Baracchini); and Agrawal et al., WO 94/01550 (Agrawal).

Lee et al., 1993

7. Lee describes an endogenous RNA transcript in *c. elegans* called lin-4, which Lee speculates may act as a natural antisense regulator of expression of a protein called lin-14. According to Lee, lin-4 is present in two forms: a short 22-nucleotide-long form (lin-4S) and a long 61-nucleotide-long form (lin-4L). Lee reports that lin-4L is a single strand that has the potential to partially hybridize to itself, while lin-4S is a single strand with little potential to form a duplex (p 848, Figure 7a).

8. Lee suggests that it is the short lin-4S that acts as a natural antisense to lin-14. The longest stretch of complementarity between lin-4 and lin-14 is 10 bases, though Lee notes that lin-4S might not include all 10 complementary bases. See page 850. Thus, lin-4S is only partially complementary to lin-14. lin-4S is a single-strand and there is no suggestion that it exists as a stable duplex. Lee represents lin-4S as an unpaired single strand (p 848, Figure 7a)

9. To arrive at a compound described in the claims starting with lin-4S as described by Lee, one would have to change the base sequence to make it fully complementary to a target. Lee provides no reason for doing that. More importantly, to arrive at a compound of the present claims, one would also have to pair such a base-modified version of lin-4S with a second, complementary strand to form a duplex. Not only does Lee fail to suggest such a duplex, Lee suggests the opposite: that the presence of a complementary strand would interfere with antisense activity. See page 850 (discussing likely inactivity of lin-4L because complementary bases are sequestered in a stem). Based on Lee, one would have no reason to create a duplex comprising a first oligonucleotide similar to lin-4S, but with a base sequence altered to be completely complementary to lin-14 and a second oligonucleotide fully complementary to the first.

10. Lee also discusses the longer transcript, lin-4L. That molecule is 61 nucleotides in length and is predicted by Lee to form a stem-loop structure. See Figure 7a. Thus, it is not a duplex consisting of two separate fully complementary strands, but a single oligonucleotide with a double-stranded portion. Lee speculates that because of this structure, lin-4L is not responsible for the observed natural antisense activity, reasoning that "the predicted secondary structure (Figure 8) would sequester within a stem most of

the bases that are complementary to lin-14, perhaps rendering lin-4 inactive for basepairing with lin-14 mRNA." See page 850. Lee further notes that "[s]ince lin-4S is more abundant than lin-4L, it seems likely that lin-4S plays the major role in basepairing with lin-14." Thus, Lee strongly suggest that because of its predicted structure and its relative low abundance compared to lin-4S, that lin-4L is not responsible for the observed activity.

11. Even if one were to assume that it is the lin-4L form that is active, despite the teaching in Lee to the contrary, there still would have been no reason to replace that single 61-nucleotide-long self-complementary molecule with a duplex comprising two separate oligonucleotides. Duplexes comprising two separate oligonucleotides and single self-complementary molecules are fundamentally different. One would recognize that if a self-complementary molecule were to function as an antisense compound, some enzyme must separate the self-complementary portion of the molecule. One cannot assume that such an enzyme would be able to likewise separate the strands of a duplex consisting of two unattached strands. Enzymes are usually highly selective for certain types of substrates. It would be incorrect to expect that an enzyme that can unwind a partially complementary hairpin would also be able to separate a fully complementary duplex composed of two unattached strands. Thus, one would be disinclined to replace the lin-4L molecule with a duplex comprising two separate strands.

12. Further, RNA is relatively unstable and susceptible to degradation by nucleases. The hairpin structure of lin-4L constrains the complementary portions of the molecule shown in Figure 7a to remain near one another, likely enhancing protection against nuclease action. The fully complementary duplexes described in the pending claims lack this hairpin and are more likely to be susceptible to nuclease action. One would be disinclined to replace a relatively stable and nuclease resistant structure with one that one would predict to be less stable and less resistant.

13. Perhaps most importantly, the hairpin lin-4L shown in Figure 7a was expressed inside a cell from an endogenous cellular gene. No data are presented suggesting that exogenous synthetic lin-4L, let alone a duplex variant containing two separate strands, could be used

to achieve the same effect. If one were to disregard the teaching of Lee that Lin-4L is not the active form and explore the possible activity of lin-4L, one might start by adding exogenous lin-4L to a cell, but there would still be no reason that one would replace that single self-complementary molecule with a duplex described in claim 120, which would be expected to be less stable, more susceptible to nuclease degradation, less likely to enter the cell, unlikely to be separated by the enzyme that unwinds lin-4L, and less likely to have activity compared to the endogenous molecule.

14. Finally, starting with either lin-4S or lin-4L one would have to include 2'-modified nucleosides arranged with RNA nucleosides in a gapmer motif to fall within the scope of the pending claims. Since lin-4S and lin-4L are endogenous molecules expressed in a cell, nothing in Lee suggests introducing modifications into a synthetic molecule. Indeed, introducing chemical alterations can disrupt the function of endogenous molecules and make them less active or inactive.

15 In summary, Lee describes certain naturally occurring transcripts. Even if one were to make synthetic versions of those transcripts, though that is not suggested in Lee, the resulting compounds would have substantial differences from those recited in the present claims. Lee does not suggest any reason for making compounds having the claimed features and in fact such compounds would have been considered ill-suited for furthering or exploiting the research described in Lee.

Manche et al.

16. Manche investigates a protein kinase called double-stranded RNA-activated inhibitor (DAI), which is activated by double-stranded RNA molecules. Manche tested whether double-stranded RNA duplexes of various lengths could activate DAI and found that short duplexes (15 and 23 base pairs) failed to bind or activate DAI. Manche speculates that duplexes shorter than 33 base pairs will not be active (page 5246). Thus, Manche does not provide a reason to make duplexes comprising oligonucleotides 17 to 25 nucleosides in length, as recited in the present claims, because duplexes of that length failed to activate DAI. Indeed, Manche specifically discourages duplexes of that length.

17. Manche also provides no reason to make such duplexes complementary to a messenger RNA, since Manche reports that complementarity of sequence is not relevant (page 5238). The duplexes in Manche are not complementary to any chosen mRNA target.

18. Further, Manche offers no reason to make chemically modified oligonucleotides. The duplexes were synthesized enzymatically, not through chemical synthesis and do not contain any chemical modifications.

19. Manche would not have prompted one to make compounds as those presently claimed and indeed, such compounds would have been unsuitable for use in the research described in Manche.

Baracchini et al.

20. Baracchini describes antisense compounds designed to activate RNase H. In that mechanism, an exogenous DNA or DNA-like oligonucleotide hybridizes to a target RNA and then RNase H cleaves the RNA strand of the resulting DNA/RNA duplex. RNase H does not cleave RNA in an RNA/RNA duplex. To function through the RNase H dependent mechanism, the compounds in Baracchini are all single stranded and all comprise at least some deoxyribonucleosides, although, as explained by Baracchini, they may also include modified nucleosides.

21. Barracchini does not provide a reason that would prompt one to make double-stranded compounds. Indeed, one would assume that such compounds would be inactive as RNase-H based antisense compounds, because the sense strand would interfere with the ability of the antisense oligonucleotide to hybridize with its intended target RNA.

22. Baracchini also does not provide a reason to make compounds comprising ribonucleosides. As noted above, ribonucleosides do not support RNase H cleavage of target RNA and would not be expected to provide any beneficial properties to the compound identified in Baracchini.

23. The present specification describes an antisense mechanism that does not depend on RNase H, and instead relies on a double-strand-specific RNase (called RNase III) to cleave double-stranded RNA. Because the mechanisms differ, it would have been impossible to make confident predictions regarding whether chemical modifications would assist or interfere with the function of RNase III. The interactions of synthetic molecules inside cells are often sensitive to even the smallest changes of molecular composition. Moreover, absent the present specification, one would not even have been prompted to consider such modifications in exploiting a double-strand specific RNase.

24. The present claims recite molecules that have features one would not have used in compounds for exploiting RNase H. Thus, in designing RNase H dependent compounds, as in Baracchini, one would not have been prompted to make compounds having the claimed features.

Agrawal et al.

25. Like Baracchini, Agrawal also describes RNase-H dependent antisense compounds, however the compounds of Agrawal have a portion of self-complementarity, which Agrawal asserts results in a molecule that is more stable. In one example, the entire oligonucleotide is self-complementary, and thus is predicted to form a double-stranded, but covalently linked compound. See compound C in Figure 5.

26. As described above, double-stranded compounds formed by a single self-complementary molecule differ fundamentally from duplexes comprising two separate molecules. They will have different stabilities towards digest by nucleases and different abilities to unwind. See e.g., paragraph 11, above. Agrawal provides no reason to make a duplex comprising two separate oligonucleotides.

27. Further, Compound C in Figure 5, which most closely resembles the presently claimed duplexes, has a duplex region that contains 12 base-pairs. If one were to replace this duplex region with two separate oligonucleotides, each oligonucleotide would be 12 nucleosides in length, well below the minimum number of 17 in the present claims.

28. Finally, as described above, the presently claimed compounds would not be considered suitable for use as RNase-H dependent antisense compounds. See Paragraph 22-23. Consequently, nothing in Agrawal would have prompted one to make such compounds.

Summary

29. None of the references individually nor all of them in the aggregate, would have prompted me, or a comparably skilled scientist in the field, to make a duplex having two separate, fully complementary oligonucleotides, where one of them is complementary to a target mRNA and has a gapmer motif comprising an RNA gap and 2'-modified nucleosides in the wings, and where each oligonucleotide is from 17-25 nucleosides in length. I would not have used such a duplex as a synthetic lin-4S as described in Lee, because lin-4S does not include a sequence that basepairs with a second strand and because it has only partial complementarity to its suspected target. I would not have made such a duplex as a substitute for lin-4L, because Lee teaches that lin-4L is not the active form of the molecule and because that compound is a long single-strand having a region of self-complementary having only partial complementarity to its suspected target. I would not have made such a duplex to activate DAI, because Manche reports that duplexes less than about 33 nucleosides in length are inactive and do not need to be complementary to mRNA. Finally, I would not have used a duplex for RNase-H based antisense as described in Baracchini or Agrawal, because I would have expected the sense strand to interfere with activity and because I would have avoided ribonucleosides. For at least these reasons, the claimed compounds are not suited to any of the uses described in the cited references.

30. I have read the office action dated February 19, 2009, and note that the office action fails even to offer a reason why one would make the claimed compounds. The office action merely remarks that short duplex RNAs in the art were synthesized for "a variety of purposes such as study of enzyme structure and regulation of gene expression." Office Action at Page 3. I have considered this remark and the office action in its entirety, but

for the reasons set forth above, I maintain that the cited references, in fact, fail to provide any reason to produce a compound described by the claims.

31. Finally, I note that on page 5, the office action asserts that "Lee et al. and Agrawal et al. teach inhibition via duplex RNAs." As explained above, those references do not teach duplexes comprising two separate oligonucleotides, as is suggested by the Examiner. Rather, those references show certain molecules having regions of self-complementarity. None of these molecules is a duplex formed by two separate oligonucleotides. Therefore, to the extent that the Examiner's assertion that the references "teach inhibition via duplex RNAs" suggests duplexes comprising separate oligonucleotides as claimed, that assertion is incorrect.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 8-05-2009

David Corey